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☐ 1. Document ID: US 6228983 B1

L8: Entry 1 of 10 File: USPT May 8, 2001

US-PAT-NO: 6228983
DOCUMENT-IDENTIFIER: US 6228983 B1

TITLE: Human respiratory syncytial virus peptides with antifusogenic and antiviral activities

DATE-ISSUED: May 8, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barney; Shawn O'Lin	Cary	NC	N/A	N/A
Lambert; Dennis Michael	Cary	NC	N/A	N/A
Petteway; Stephen Robert	Cary	NC	N/A	N/A

US-CL-CURRENT: 530/300; 424/186.1, 424/211.1, 530/324, 530/325, 530/326

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6190702 B1

L8: Entry 2 of 10 File: USPT Feb 20, 2001

US-PAT-NO: 6190702
DOCUMENT-IDENTIFIER: US 6190702 B1

TITLE: Sustained-released material prepared by dispersing a lyophilized polypeptide in an oil phase

DATE-ISSUED: February 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Takada; Shigeyuki	Kobe	N/A	N/A	JPX
Kurokawa; Tomofumi	Kawabe-gun	N/A	N/A	JPX
Iwasa; Susumu	Tsuzuki-gun	N/A	N/A	JPX

US-CL-CURRENT: 424/501; 424/451, 424/457, 424/489, 424/499, 514/963, 514/964

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6025194 A

L8: Entry 3 of 10 File: USPT Feb 15, 2000

US-PAT-NO: 6025194
DOCUMENT-IDENTIFIER: US 6025194 A

TITLE: Nucleic acid sequence of senescence asssociated gene

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Funk; Walter	Hayward	CA	N/A	N/A

US-CL-CURRENT: 435/320.1; 435/325, 536/23.1, 536/23.5, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 6017536 A

L8: Entry 4 of 10 File: USPT Jan 25, 2000

US-PAT-NO: 6017536
DOCUMENT-IDENTIFIER: US 6017536 A

TITLE: Simian immunodeficiency virus peptides with antifusogenic and antiviral activities

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barney; Shawn O'Lin	Cary	NC	N/A	N/A
Lambert; Dennis Michael	Cary	NC	N/A	N/A
Petteway; Stephen Robert	Cary	NC	N/A	N/A
Langlois; Alphonse J.	Durham	NC	N/A	N/A

US-CL-CURRENT: 424/188.1; 424/208.1, 530/300, 530/324, 530/325, 530/326

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 5. Document ID: US 6008339 A

L8: Entry 5 of 10 File: USPT Dec 28, 1999

US-PAT-NO: 6008339
DOCUMENT-IDENTIFIER: US 6008339 A

TITLE: Nucleic acids encoding a neural tissue affecting factor

DATE-ISSUED: December 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harland; Richard M.	Berkeley	CA	N/A	N/A
Smith; William C.	Oakland	CA	N/A	N/A

US-CL-CURRENT: 536/23.51; 435/252.3, 435/320.1, 435/325, 435/69.4, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw Desc | Image

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☐ 6. Document ID: US 5885829 A

L8: Entry 6 of 10 File: USPT Mar 23, 1999

US-PAT-NO: 5885829
DOCUMENT-IDENTIFIER: US 5885829 A

TITLE: Engineering oral tissues

DATE-ISSUED: March 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mooney; David J.	Ann Arbor	MI	N/A	N/A
Rutherford; Robert B.	Ann Arbor	MI	N/A	N/A

US-CL-CURRENT: 435/325; 424/422, 424/435, 424/49, 435/374, 435/378, 435/69.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw Desc | Image

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☐ 7. Document ID: US 5739107 A

L8: Entry 7 of 10 File: USPT Apr 14, 1998

US-PAT-NO: 5739107
DOCUMENT-IDENTIFIER: US 5739107 A

TITLE: Morphogen treatment of gastrointestinal ulcers

DATE-ISSUED: April 14, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Charles M.	Medway	MA	N/A	N/A
Charette; Marc F.	Needham	MA	N/A	N/A
Kuberasampath; Thangavel	Medway	MA	N/A	N/A
Rueger; David C.	Hopkinton	MA	N/A	N/A
Oppermann; Hermann	Medway	MA	N/A	N/A
Pang; Roy H. L.	Etna	NH	N/A	N/A
Ozkaynak; Engin	Milford	MA	N/A	N/A
Smart; John E.	Weston	MA	N/A	N/A

US-CL-CURRENT: 514/12; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 5693615 A

L8: Entry 8 of 10 File: USPT Dec 2, 1997

US-PAT-NO: 5693615
DOCUMENT-IDENTIFIER: US 5693615 A

TITLE: Therapeutic compositions for osteoinduction

DATE-ISSUED: December 2, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stone; Roger Lee	Hamilton	OH	N/A	N/A

US-CL-CURRENT: 514/12; 514/167, 514/21, 530/350, 530/840

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 9. Document ID: US 5520923 A

L8: Entry 9 of 10 File: USPT May 28, 1996

US-PAT-NO: 5520923
DOCUMENT-IDENTIFIER: US 5520923 A

TITLE: Formulations for delivery of osteogenic proteins

DATE-ISSUED: May 28, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tjia; Jane S.	Malden	MA	N/A	N/A
Kelley; Brian D.	Medford	MA	N/A	N/A
Northey; Richard P.	Ipswich	MA	N/A	N/A
Philbrook; C. Michael	Boston	MA	N/A	N/A

US-CL-CURRENT: 424/426; 264/321, 528/495

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 10. Document ID: US 5385887 A

L8: Entry 10 of 10 File: USPT Jan 31, 1995

US-PAT-NO: 5385887
DOCUMENT-IDENTIFIER: US 5385887 A

TITLE: Formulations for delivery of osteogenic proteins

DATE-ISSUED: January 31, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yim; Kalvin W. K.	N. andover	MA	N/A	N/A
Huberty; Michael C.	Andover	MA	N/A	N/A
Northey, Jr.; Richard P.	Ipswich	MA	N/A	N/A
Schrier; Jay A.	Andover	MA	N/A	N/A

US-CL-CURRENT: 514/12; 106/645, 424/423, 424/426, 514/21, 514/8, 530/350, 530/397, 530/399, 530/840

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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CARTILAGE.USPT.	6268
CARTILAGES.USPT.	422
(7 AND (CARTILAGE OR BONE)).USPT.	10

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L8: Entry 6 of 10 File: USPT Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885829 A
TITLE: Engineering oral tissues

BSPR:
Gingival samples are contemplated for use in the present invention for the regeneration of dental pulp, dentin, periodontal tissue, and bone, as well as oral submucosa and gingival submucosa (subgingival connective tissue). Using a gingival tissue sample means that a tooth does not need to be extracted in order to obtain pulp or periodontal ligament fibroblasts as a source of cells. Gingival biopsies are obtainable by routine dental procedures with little or no attendant donor site morbidity.

BSPR:
The use of gingival cells is currently preferred for the induction of mineralized oral connective tissues, such as dentin, cementum and bone. However, the generation of muscle cells as part of the tongue regeneration is not excluded as the gingival fibroblasts may be engineered to differentiate into muscle cells.

BSPR:
Further examples of appropriate synthetic matrices are polyanhydrides, polyesters, polyorthoesters, and poly(amino acids), polypeptides, polyethylene oxide, polyphosphazenes, various block copolymers, such as those consisting of ethylene oxide and propylene oxide (e.g., Pluronic surfactant; BASF Corp.), and blends of polymers from this group and with other polymers. Further polymers are detailed herein in Example IV. Ceramics, such as calcium phosphate matrices, may also be employed in the present invention.

BSPR:
The present invention is generally applicable to the culture and regeneration of a variety of oral tissues and structures. By way of example only, one may mention dental pulp tissue, dentin, periodontium, bone, cementum, gingival submucosa, oral submucosa, salivary gland tongue and taste bud tissues.

BSPR:
The use of gingival samples is contemplated to be advantageous as this can give rise to regenerated tissues of dental pulp, dentin, periodontium, cementum, bone, oral submucosa, gingival submucosa, and even has the potential to regenerate striated muscle cells.

BSPR:
Dental pulp, oral and gingival submucosal tissues are advantageous as they are constitutively capable of tissue regeneration. However, the use of viable cells isolated from the oral or gingival submucosa in the regeneration of other tissues, such as dental pulp, dentin, cementum, periodontal ligament and bone, will be understood to involve, in certain embodiments, the induction of particular developmental pathways by, e.g., the addition of specific protein factors or exposure to particular conditions. Genetic engineering to induce, promote or assist in the development of a particular tissue is also contemplated.

DEPR:
In many situations, damage to oral tissues cannot be effectively repaired. This leads to alternations in the form and function of these tissues. Such alterations may give rise to deformities of the face, jaws and teeth which may be disfiguring as well as disabling. Loss of teeth leads to collapse of the dental arch and malpositioning of remaining teeth. Malpositioning may increase susceptibility to diseases such as caries, gingivitis, and periodontitis which in turn can lead to additional tooth loss. Tooth loss leads to diminished mastication, and eventually to a diminution in the size of the jaw bones rendering prosthetic reconstruction more difficult. It will be understood that oral tissue damage is painful to the individual and costly to society.

DEPR:
Fibroblasts or undifferentiated mesenchymal cells also have an important role in wound healing mechanisms in the pulp. The fibroblasts of the cell-rich zone are thought to differentiate into odontoblasts after the right stimulus--for example, growth factor, a bone morphogenic protein (BMP), cytokine, or inflammatory mediator, released during wounding from the exposed predentin or dentin, or inflammatory cells that have migrated to the wound site.

DEPR:
The outer surface of the root is covered by a relatively thin layer of a bone-like mineralized tissue called cementum. Cementum consists of a matrix of calcified collagenous fibrile, glycoproteins, and mucopolysaccharides. The outermost layer of cementum is an uncalcified precementum produced by the discontinuous layer of irregularly shaped cementoblasts.

DEPR:
The lamina propria of the gingiva is firmly attached to the periosteum of the alveolar bone except as it approaches to within 1 or 2 mm of the crown surface. This narrow band of gingiva surrounding each tooth is called the "free gingiva" as opposed to the rest of the gingiva, which is "attached" to the alveolar bone.

DEPR:
Another embodiment of the present invention utilizes viable

cells isolated from oral or gingival submucosa to regenerate other tissues such as dental pulp, dentin, cementum, periodontal ligament and bone. Such cells propagated in vitro, seeded onto scaffold and developed in vitro, would be constitutively capable, genetically engineered or induced by the addition of specific protein factors to produce the specific tissue of interest when implanted in vivo.

DEPR:
Calcium phosphate ceramics are non-biodegradable matrices that are extensively used in engineering bone tissue (Ducheyne, 1988) and may be used in the present invention. A suitable ceramic that may be used is described in U.S. Pat. No. 4,596,574, incorporated herein by reference.

DEPR:
Both hydroxyapatite and tricalcium phosphate, and mixtures of the two, may be utilized. These materials can be coated over metal implants (Lemons, 1988), used with the tissue implant as an additional bone inductive or conductive material (Ducheyne, 1988; Jarcho, 1981), or used as a cell delivery vehicle (Goshima et al., 1991). These materials only release calcium and phosphate as breakdown products. They display no local or systemic toxicity, and become directly bonded to adjacent bone tissue with no intervening fibrous capsule (Ducheyne, 1988). The erosion and mechanical properties of these materials are controlled by the specific chemical composition and processing conditions (Lemons, 1988).

DEPR:
Bone achieves its high mechanical moduli by combining organic and inorganic materials, and this principle is being utilized to synthesize new ceramic materials. Apatite crystals are being synthesized by nucleation and growth around poly(amino acids) (Stupp and Ciegler, 1992). This results in an intimate dispersion of the organic molecules within the ceramic, and improves the mechanical properties of the ceramic (Stupp et al., 1993). This process may mimic the process of natural bone formation, and these materials show promise in engineering bone tissue (Stupp et al., 1993). Such materials may also be used in aspects of this invention.

DEPR:
An exemplary, but by no means limiting, list of markers which are contemplated for use in the present invention includes: human gene markers for fibrous connective tissue (produced by either pulp or gingival fibroblasts) for cellular fibronectin or collagen type I, and III, measurable as protein or RNA; human gene markers for fibrous connective tissue (produced by either pulp or gingival fibroblasts) for BMP receptors BMPR-A1, -AII, -II or Act (activin) RI, BMP-2, -4 or -7, or MSX-2, a homeobox containing transcription factor which has been implicated as a mediator of BMP signals during tissue (including tooth) development, measurable as RNA and perhaps necessary to be responsible to BMP induction of bone or dentin formation; and alkaline phosphatase enzyme activity, which is associated with cells such as osteoblasts and odontoblasts

involved in mineralized tissue formation.

DEPR:
Additional markers include dentinsialposphophoryn (DSPP), a putative specific marker for dentin, and bone sialoprotein, osteonectin and osteocalcin, which are highly enriched in bone. Markers for use for the buccal mucosa include cytokeratins 4 and 13. Further, markers for use in identification of taste buds include cytokeratins 8, 18 and 19, which are present in taste buds but absent in the surrounding mucosa.

DEPR:
Mechanical signals are known to regulate the development of a variety of tissues, including muscle (Vandenburgh et al., 1991), and bone (Carter et al., 1989). For example, engineered tendons that are not subjected to mechanical loading do not develop mechanical moduli as high as normal tendons, even though they appear to be histologically identical (Cao et al., 1994). Mechanical stimuli (e.g., strain, shear) also clearly regulate the gene expression of cultured cells (Frangos, 1993). To engineer an optimally functional oral tissue it may be necessary to provide the correct mechanical stimuli during the process of tissue development.

DEPR:
BMP proteins may be employed in certain aspects of the present invention, such as those described in U.S. Pat. Nos. 4,795,804; 4,877,864; 4,968,590; 5,011,691; 5,013,649; 5,106,748; 5,108,753; 5,116,738; 5,141,905; 5,166,058 and 5,187,076, each incorporated herein by reference. For example, the inventors have demonstrated that a single application of BMP-7 to a freshly and partially amputated dental pulp induced reparative dentinogenesis in ferrets, monkeys and humans (Rutherford et al., 1993b, 1994, 1995). Additionally, the inventors have demonstrated that BMP-7 induced bone when implanted in gingiva, indicating that gingiva possess cells that are capable of forming mineralized tissue such as bone.

DEPR:
Direct local application of recombinant growth factors (e.g., BMP-2) has been shown to induce reparative dentinogenesis in dogs and primates when placed on partially amputated dental pulps (Rutherford et. al., 1993b; Nakashima, 1994; Rutherford et. al., 1994), or on a freshly cut dental surface ("transdentinal" application; Rutherford et. al., 1995). However, in many clinical situations no pulp remains to stimulate. The present invention provides new, preferably autologous, pulp tissue to replace lost pulp tissue and form reparative dentin.

DEPR:
Aliphatic polyesters of the poly(.alpha.-hydroxy acids) have the general formula --[--O--CH(R)--CO--]-- which derive from corresponding HO--CH(R)--COOH where R.dbd.H in the case of glycolic acid (GA) and R.dbd.CH3 in the case of lactic acid (LA), the latter being chiral, i.e., D- or L-isomer is possible. These polymers have been used in bone osteosynthesis

and reconstruction (Vert et al., 1984) and in drug delivery (Gombotz and Pettit, 1995).

DEPR:
PVA gels can be prepared by cross-linking with formaldehyde in the presence of sulfuric acid (Schwartz et al., 1960). These formaldehyde-cross-linked PVA materials have been used as prosthesis for a variety of plastic surgery applications including breast augmentation (Clarkson, 1960 and Peters and Smith, 1981), diaphragm replacement (Haupt and Myers, 1960) and bone replacement (Camerson and Lawson, 1960). However, a variety of complications were found after long term implantation, including calcification of the PVA (Peters and Smith, 1981).

DEPR:
Pluronic polyols or polyoxamers are block copolymers of PEO and poly(propylene oxide and are usually synthesized by anionic polymerization in the form of a ABA triblock using a difunctional initiator (Schmolka, 1972). Pluronics F 127, which contains 70% ethylene oxide and 30% propylene oxide by weight with an average molecular weight of 11,500, is the most commonly used gel-forming polymer matrix to deliver proteins (Gombotz and Pettit, 1995).

DEPR:
To infiltrate sponges with PVA (Aldrich Chem. Co.; Milwaukee, Wis.; MW 3000, 75% hydrolyzed) or the Pluronic F 108 surfactant (BASF; Parsippany, N.J.), sponges were immersed for 16 hr in an aqueous solution containing 1-100 mg/mL of PVA or Pluronic in phosphate buffered saline (PBS). The sponges were subsequently removed from the solution, dried, and lyophilized. The mass of devices before and after coating was quantitated to determine the mass of incorporated PVA or surfactant. To determine whether the incorporated PVA was permanently associated with the sponges, some sponges were subsequently soaked in a solution of PBS overnight, air dried at room temperature, lyophilized, and reweighed. All sponges were sterilized before use by exposure to ethylene oxide.

DEPR:
Discs (0.5 mm thick) were formed from 0.75 g of PLA, polyglycolic acid, poly-D,L-lactic acid, or a 85/15 or 50/50 copolymer of lactic and glycolic acid (all purchased from Medisorb) using a Carver Laboratory Press (Fred S. Carver, Inc.; Menominee Falls, Wis.). The polymer was heated to 185.degree. C., and compressed at 1500 psi. Discs were coated with PVA or Pluronic F108 as described above. Contact angle measurements were made from an advancing water droplet using a goniometer (Rame-Hart, Inc.; Mountain Lakes, N.J.). Reported values represent the mean and standard error of the mean (SEM) calculated from the mean advancing contact angle of a minimum of three films at each condition. The mean advancing contact angle for each film was calculated from a minimum of three measurements.

DEPR:

Polymers of the lactic/glycolic acid family are all relatively hydrophobic, as indicated by high contact angles with water. The contact angle of films fabricated from poly-L, lactic acid, polyglycolic acid, poly-D,L lactic acid, and 85/15 and 50/50 copolymers of lactic and glycolic acid were 79.degree..+-.2.degree., 73.degree..+-.2.degree.,72.degree..+-.1.degree., 73.degree..+-.2.degree., and 69.degree..+-.3.degree., respectively. To determine whether the hydrophobicity of these polymers could be decreased, solid films of PLA were coated with the hydrophilic polymer PVA or a surfactant, Pluronic F 108.

DEPR:
The advancing water contact angle decreased from 79.degree..+-.2.degree. to 23.degree..+-.2.degree. when devices were exposed to PBS containing 10 mg/mL solution of PVA. When PVA solutions ranging from 1 to 100 mg/mL were used for coating, a similar decrease in the contact angle of polymer films was noted. The contact angle of PLA films also decreased from 79.degree..+-.2.degree. to 22.degree..+-.3.degree. when a 1 mg/mL solution of the Pluronic surfactant was utilized in place of the PVA.

DEPR:
Contact angle decreases in the same range were found when devices fabricated from PGA, or copolymers of lactic and glycolic acid were similarly treated with PVA (e.g., the contact angle of films of a 50/50 copolymer decreased to 21.degree..+-.7.degree.). The decrease in the hydrophobicity of the polymer films was not permanent, however, as immersion of coated polymer discs into a PBS solution led to a rebound in the contact angle over a 24 hr period. For example, the contact angle of Pluronic-coated devices returned to 76.degree..+-.2.degree. after 24 hr in PBS. These results suggest that the coating molecule re-dissolved over time in an aqueous environment.

DEPR:
This method for fabricating devices has wide applicability. A variety of other types of water soluble molecules, such as the Pluronic surfactants, can also be utilized as the coating molecule. This type of treatment will be useful for improving cell seeding into a variety of hydrophobic polymer devices, both biodegradable and non-biodegradable.

DEPR:
The cell/scaffold constructs comprised of human pulp and gingival derived fibroblasts expressed the following genes associated with the development of bone and dentin; bone morphogenetic proteins -2, -4 and -7, bone morphogenetic protein receptors -IA, -IB and -II, activin receptor -1, type I collagen, and the transcription factor MSX-2, both before and after implantation in vivo. Light microscopic examination of cell/scaffold constructs comprised of human pulp and gingival derived fibroblasts incubated in vivo for 3 weeks revealed masses of tissue comprised of residual scaffold with attached

cells, interspersed with unattached cells, connective tissue matrix and patent blood vessels which penetrate the mass. Analysis of RNA extracted from pulp and gingival fibroblast/PGA constructs by the reverse transcriptase polymerase chain reaction revealed the presence of human specific RNA for Alu sequences indicating that human cells had survived in vivo, whereas control implants without cells or containing mouse cells failed to express these human specific genes. Immunocytochemical analyses revealed that, whereas most of the fibrous connective tissue contained within the residual PGA fibers was of mouse origin, small amounts of human fibronectin was present.

DEPU:
Camerson and Lawson, "The failure of polyvinyl sponge as a bone substitute," Res. Vet. Sci., 1:230-231, 1960.

DEPU:
Carter et al., "Relationships between loading history and femoral cancellous bone architecture," J. Biomech., 22:231-244, 1989.

DEPU:
Goshima et al., "The origin of bone formed in composite grafts of porous calcium phosphate ceramic loaded with marrow cells," Clin. Orthopod. Rel. Res., 269:274-283, 1991.

DEPU:
Kim et al., "Fluorometric Assay of DNA in Cartilage Explants Using Hoechst 33258," Anal. Biochem., 174:168-176, 1988.

DEPU:
Morikawa et al., "Enhancement of therapeutic effects of recombinant interleukin-2 on a transplantable rat fibrosarcoma by the use of a sustained release vehicle, pluronic gel," Cancer, 47:37-41, 1987.

DEPU:
Nakashima, "Induction of Dentin Formation on Canine Amputated Pulp by Recombinant Human Bone Morphogenetic Proteins (BMP)-2 and -4," J. Dent. Res., 73:1515-1522, 1994.

DEPU:
Pautian et al., "Intravenous Pluronic F-127 in early burn wound treatment in rats," Burns, 19:187-191, 1993.

DEPU:
Schmolka, "Artificial skin. I Preparation and properties of pluronic F-127 gels for treatments of burns," J. Biomed. Mater. Res., 6:571-582, 1972.

DEPU:
Stupp and Ciegler, "Organoapatites: materials for artificial bone. I. synthesis and microstructure," J. Biomed. Matls. Res., 26:169-183, 1992.

DEPU:

Stupp et al., "Organoapatites: materials for artificial bone. II. hardening reactions and properties," J. Biomed. Matls. Res., 27:289-299, 1993a.

DEPU:
Vacanti et al., "Tissue engineered growth of new cartilage in the shape of a human ear using synthetic polymers seeded with chondrocytes." In: Tissue inducing biomaterials, Cima and Ron, eds., Mat. Res. Soc. Symp. Proc., 252:367-374, 1992.

DEPU:
Vert et al., "Bioresorbable plastic materials for bone surgery," In: Macromolecular Biomaterials. Hastings and Ducheyne, eds., CRC Press, Boca Raton, Fla., 1984.

DEPW:
d. Pluronics

ORPL:
Goshima et al., "The origin of bone formed in composite grafts of porous calcium phosphate ceramic loaded with marrow cells," Clin. Orthopod. Rel. Res., 269:274-283, 1991.

ORPL:
Gu, et al., "Expression of genes for bone morphogenetic proteins and receptors in human dental pulp", Archives of Oral Biology, 41(10):919-23, 1996.

ORPL:
Nakashima, "Induction of Dentin Formation on Canine Amputated Pulp by Recombinant Human Bone Morphogenetic Protieins (BMP)-2 and -4," J. Dent Res., 73:1515-1522, 1994.

ORPL:
Suwa et al., "Inductive Effect of Bovine Bone Morphogenetic Protein on Human Dental Pulp Tissue In Vitro," 13-Mammalian Biochem., 122:707, 1995, Abstract No. 77691w.

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L8: Entry 8 of 10 File: USPT Dec 2, 1997

DOCUMENT-IDENTIFIER: US 5693615 A
TITLE: Therapeutic compositions for osteoinduction

ABPL:
A method for generating new bone growth in a mammal comprising administrating to the mammal a safe and effective amount of a Vitamin D compound in combination with a safe and effective amount of osteoinductive extract or at least one BMP.

BSPR:
The present invention relates to the field of osteoinduction (bone growth). Specifically, the present invention relates to novel therapeutic formulations comprising the administration of bone morphogenetic proteins and a Vitamin D compound, resulting in synergistic bone growth.

BSPR:
In healthy individuals bone growth generally proceeds normally and fractures heal without the need for pharmacologic intervention. Nonetheless, in certain instances bones may be weakened or may fail to heal properly. For example, healing may proceed slowly in the elderly and in patients undergoing treatment with corticosteroids, such as transplant patients and those being treated for chronic lung disease. Another example is osteoporosis. Osteoporosis is an abnormal loss of bony tissue often occurring in post-menopausal woman and elderly men. The disorder increases the risks of small fractures occurring in the bones, particularly the spine. At present, osteoporosis is treated mainly by supplements of calcium, vitamin D, estrogen, or calcitonin, a hormone which controls the body's use of calcium. Unfortunately, these treatments are merely preventative against the further loss of bone. There is a need in the art for treatments that go beyond the prevention of bone loss and promote bone formation and/or reverse bone loss.

BSPR:
(1989) "Bone Morphogenic Proteins and Vitamin D", Nutrition Reviews, Vol. 47, pp. 364-366 concludes that Vitamin D in the diet prevents the loss of the osteoinductive activity of bone matrix.

BSPR:
Turner, R. T., J. Farley, J. J. Vandersteenhoven, S. Epstein, N. H. Bell, and D. J. Baylink, (1988) "Demonstration of Reduced Mitogenic and Osteoinductive Activities in Demineralized

Allogeneic Bone Matrix from Vitamin D-deficient Rats", The Journal of Clinical Investigation, Inc., Vol. 82, pp. 212-217, discloses the implantation of demineralized bone matrix from Vitamin D-deficient rats into normal rats. The demineralized bone matrix from Vitamin D-deficient rats did not promote osteoinduction as effectively as demineralized bone matrix from normal rats.

BSPR:
Sampath, T. K., S. Weintraub, and A. H. Reddi, (1984) "Extra-cellular Matrix Proteins Involved in bone Induction are Vitamin D Dependent", Biochemical and Biophysical Research Communications, Vol. 124, pp. 829-835, discloses a study involving implantation of demineralized bone matrix from normal rats and demineralized bone matrix from rachitic rats wherein the rachitic bone matrix did not induce bone growth while the normal bone matrix did. The study concluded that these results demonstrate that Vitamin D is necessary to produce bone inductive proteins in the bone matrix of a living rat.

BSPR:
U.S. Pat. No. 4,761,471, Urist, assigned to the Regents of the University of California, issued Aug. 2, 1988, discloses a bone morphogenetic protein composition comprising BMP factor and BMP associated protein having a molecular weight of 34,000 daltons. Use of such factors and compositions to induce bone formation in mammals is also disclosed.

BSPR:
U.S. Pat. No. 4,455,256, Urist, assigned to the Regents of the University of California, issued Jun. 19, 1984, discloses a bone morphogenetic protein having a molecular weight in the range of 1,000 to 100,000 daltons.

BSPR:
Various other bone morphogenetic proteins/factors, osteoinductive factors, osteogenic factors and other proteins/factors related to bone growth are disclosed in the following publications: U.S. Pat. No. 4,968,590, Kubersampath and Rueger, issued Nov. 6, 1990; U.S. Pat. No. 4,698,328, Neer, Potts and Slovik, issued Oct. 6, 1987; U.S. Pat. No. 4,877,864, Wang, Wozney and Rosen, issued Oct. 31, 1989; U.S. Pat. No. 4,861,757, Antoniades, Lynch and Williams, issued Aug. 29, 1989; U.S. Pat. No. 4,810,691, Seyedin, Thomas, Bentz, Ellingsworth and Armstrong, issued Mar. 7, 1989; U.S. Pat. No. 4,804,744, Sen, issued Feb. 14, 1989; U.S. Pat. No. 4,795,804, Urist, issued Jan. 3, 1989; U.S. Pat. No. 4,789,663, Wallace, Smestad, McPherson, Piez and Ross, issued Dec. 6, 1988; U.S. Pat. No. 4,789,732, Urist, issued Dec. 6, 1988; U.S. Pat. No. 4,774,322, Seyedin, Thomas, Bentz, Ellingsworth and Armstrong, issued Sep. 27, 1988; U.S. Pat. No. 4,698,328, Neer and Slovik, issued Oct. 6, 1987; U.S. Pat. No. 4,627,982, Seydin and Thomas, issued Dec. 9, 1986; U.S. Pat. No. 4,619,989, Urist, issued Oct. 28, 1986; U.S. Pat. No. 4,596,574, Urist, issued Jun. 24, 1986; U.S. Pat. No. 4,563,489, Urist, issued Jan. 7, 1986; U.S. Pat. No. 4,563,350, Nathan, Seyedin and Bentz, issued Jan. 7, 1986; U.S. Pat. No. 4,526,909, Urist, issued

Jul. 2, 1985; U.S. Pat. No. 4,434,894, Seyedin and Thomas, issued Feb. 23, 1984; U.S. Pat. No. 4,294,753, Urist, issued Oct. 13, 1981; European Patent Application 349 048, Bab, Muhlrad, Gazit and Shteyer, published Jan. 3, 1990; European Patent Application 309 241, Chu, Nathan and Seyedin, published Mar. 29, 1989; European Patent Application 336 760, Bentz, Nathan, Rosen, Dasch and Seyedin, published Oct. 11, 1989; European Patent Application 145 155, Sen, published Jul. 10, 1985; World Patent Application 89/10934, Roos, Burns, Guy and McKnight, published Nov. 16, 1989; World Patent Applications 89/09787 and 89/09788, Oppermann, Kubersampath, Rueger and Ozkaynak, published Oct. 19, 1989; and World Patent Application 88/00205, Wang, Wozney and Rosen, published Jan. 14, 1988.

BSPR:
It is an object of the present invention to provide a method for generating new bone growth in a mammal.

BSPR:
It is a further object of the present invention to provide a pharmaceutical composition which can be used to generate new bone growth in a mammal.

BSPR:
The present invention relates to a method of generating new bone growth in mammals comprising administration to a mammal a combination of a safe and effective amount of a Vitamin D compound, and a safe and effective amount of one or more BMPs or osteoinductive extract comprising one or more BMPs.

BSPR:
The present invention further relates to a composition for generating new bone growth in mammals comprising a safe and effective amount of a Vitamin D compound; a safe and effective amount of a BMP or osteoinductive extract comprising one or more BMPs; and a pharmaceutically-acceptable carrier.

BSPR:
The present invention comprises the administration to a mammal of a combination of a safe and effective amount of a Vitamin D compound and a safe and effective amount of one or more BMPs or an osteoinductive extract comprising one or more BMPs. It has been determined that treatment with a Vitamin D compound, BMP or osteoinductive extract alone increases bone growth. Surprisingly, it has been further determined that treatment with a Vitamin D compound in combination with osteoinductive extract or in combination with at least one BMP results in a level of new bone growth greater than that achieved through administration of the BMP, osteoinductive extract or Vitamin D compound alone. Subjects in need of such treatment suffer from a variety of ailments which may be treated via this procedure, including but not limited to, bone fractures (closed and open), non-union fractures, congenital defects, as an adjunct in plastic surgery, in treating oncological resections, all diseases classified as osteoporosis, rheumetoid arthritis, osteoarthritis, septic arthritis, rickets, organic incorporation of prosthetic joints and dental implants,

periodontal disease and defects, as well as osteopenic and osteomalacic conditions and disease.

BSPR:
As used herein, "fracture reduction" means the restoration of a bone fracture by surgical or manipulative means to its normal anatomical relation.

BSPR:
As used herein, "BMP" means bone morphogenetic protein.

BSPR:
As used herein "regional treatment" includes treating bone fractures (closed and open), treating non-union fractures, treating congenital defects, as an adjunct treatment to plastic surgery, treating oncological resections, organic incorporation of prosthetic joints, organic incorporation of dental implants, and treatment of periodontal disease and defects.

BSPR:
As used herein, "mineralized tissue" means bone and teeth.

BSPR:
Bone Morphogenetic Proteins

BSPR:
In one embodiment of the present invention, a Vitamin D compound is administered in combination with one or more BMPs to generate new bone growth in a mammal. These BMPs are preferably selected from the group consisting of BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7.

BSPR:
A safe and effective amount of a BMP, preferably selected from the group consisting of BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7, is dosed in combination with a Vitamin D compound.

BSPR:
As used herein, "BMP-2" means a peptide encoded by a DNA sequence comprising SEQ ID NO:2. The DNA sequence encoding BMP-2 has ATCC No. 40345 (see ATCC/NIH REPOSITORY CATALOGUE). Isolation of BMP-2 is disclosed in U.S. Pat. No. 5,013,649, Wang, Wozney and Rosen, issued May 7, 1991; U.S. Pat. No. 5,166,058, Wang, Wozney and Rosen, issued Nov. 24, 1992; and U.S. Pat. No. 5,168,050, Hammonds and Mason, issued Dec. 1, 1992; each of which is incorporated herein by reference. Preferably the ratio of BMP-2 to Vitamin D dosed is from about 1:83 to about 1:167. In one embodiment of the present invention, 500 ng of BMP-2 is dosed with 6 ng of 1,25 dihydroxy Vitamin D.sub.3. In another embodiment of the present invention, 1000 ng of BMP-2 is dosed with 6 ng of 1,25 dihydroxy Vitamin D.sub.3.

BSPR:
Another component of the invention is an osteoinductive extract. As used herein, "osteoinductive extract" means a

chemical extract of bone, comprising one or more various bone morphogenetic proteins, including, but not limited to, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7, wherein each BMP has a molecular weight of from about 28,000 to about 40,000 daltons.

BSPR:
Snip the skin at the ankles of a 7-8 week old Long-Evans rat (Charles River laboratories, Wilmington, Mass.). Remove both tibiae and place in cold water. Rinse the bone with distilled water to remove non-osseous tissue (tissue other than bone). Allow the bone to air dry. Grind the bones by placing in an Osterizer (Oster Commercial, Milwaukee, Wis.) blender with water and ice. With the blender set at "liquefy" speed, continue to add bone. Allow the blended material to settle for a few minutes. Decant the liquid layer. Place the solid layer on a stirring plate and add distilled water to wash. Continue washing until the distilled water washes clear. Once the distilled water is clear, add ice and stir. Add 1 ml of 1 mM of phenylmethylsulfonyl fluoride (PMSF). Wash for 1 hour adding ice frequently. Repeat with a second water wash. Place the sample in an ice water bath on a stirring plate. Defat with absolute ethanol, then defat twice with ethyl ether. Spread bone material onto glass petri dishes. Allow the bone chips to air dry overnight.

BSPR:
Weigh the bone chips following the overnight drying, Using a sieve (U.S.A. Standard Sieve Series, Newark Wire Cloth Co., Newark, N.J.; sieve #40 retains particles greater than 425 mm and sieve #170 retains particles greater than 90 mm), isolate the bone particles in the 90-425 um range. Grind any particles greater than 425 mm in a MicroMill (Scienceware Bel-Art Products, Pequannock, N.J.) for 1 minute adding dry ice to the bone particles to keep the material cold. Repeat the sieving and MicroMill grinding steps of the greater than 425 mm particles until the amount of total recovery is greater than 2/3 of the initial weight of the bone. Store the particles at 4.degree. C. until the next step. Weigh the particles isolated thus far. For each gram of particles, add 25 ml of 0.6N HCl. Stir vigorously at 4.degree. C. for 2 hours. After 2 hours, stop stirring and allow the particles to settle. Decant the HCl. Add fresh 0.6N HCl and stir again for 2 hours. Decant the HCl and add fresh 0.6N HCl a third time and stir for two hours. Decant the HCl and rinse with distilled water. Using litmus paper, check the pH of the water for the presence of HCl. Continue rinsing with distilled water until the pH is between about 5 and 5.5. Rinse the bone particles with ethanol three times. Swirl, allow to settle, and remove the supernatant. Rinse the bone particles with ethyl ether three times as above. Dry overnight in glass plates. The dried bone particles are referred to as "acid demineralized bone particles".

BSPR:
The acid demineralized bone particles are deproteinized as follows: Weigh the material following the overnight drying. For each gram of material, add a solution of 30 ml 4M

guanidine-HCl, 10 mM Tris and 1.0 mM PMSF pH 6.4 to the bone material in a beaker. Extract for 16 hours at 4.degree. with vigorous stirring. Following the 16 hour extraction, cease stirring and allow the material to settle. Pour off the guanidine solution and save. Extract the material a second time for 6-7 hours using fresh guanidine-HCl solution. Following the extraction, pour off the solution and combine with the previously saved solution. The bone particles are now demineralized and deproteinized.

BSPR:
The pooled CM-Sepharose fractions are dialyzed three times for 24 hours each against 1% acetic acid. The dialysate is lyophilized to dryness and the protein pellet dissolved into 30 ml of 6M urea, 0.5M NaCl, 25 mM Na phosphate, pH 7.4. The sample is applied on a column of chelating Sepharose charged with zinc and equilibrated with the above buffer. The column is washed with the above buffer and then eluted with a gradient from 6M urea, 0.5M NaCl, 25 mM Na phosphate, pH 7.4 to 6M urea, 0.5M NaCl, 25 mM Na acetate, pH 4.6. Aliquots of each fraction are labeled with 125I and analyzed by SDS gel electrophoresis. Aliquots (100 ml) of each fraction are combined with 400 ml of elution buffer, dialyzed against 1% acetic acid and assayed for activity. Highly purified molecular weight range (M.sub.r) 25-40 kD peptides are assayed in the bone induction assay.

BSPR:
Additional examples of carriers include collagen, demineralized bone particles, ceramic and metallic implant materials, collagen membrane and bone grafts (isogenic or allogenic).

BSPR:
The active components of the present invention are also useful when injected. The dosage of the active components of the present invention which is both safe and effective to provide bone growth activity will vary with the particular condition being treated, the severity of the condition, the duration of treatment, the specific mixture of compounds employed and its usage concentration, and like factors within the specific knowledge and expertise of the attending physician and commensurate with a reasonable benefit/risk ratio associated with the use of any drug compound. In addition, lower dosages will be utilized when only local or minor bone growth is desired, whereas higher dosages will be utilized when general or major bone growth is desired.

BSPR:
The aqueous solutions preferably consist of water (preferably from about 80% to about 99.999%), a suitable solubilizer, various types of acids, and an antimicrobial agent. Several solubilizers are known. Examples of such solubilizers are as follows: urea compounds (e.g., urea; urethan); surfactants (e.g., TWEENS.RTM.; Spans; sodium deoxycholate and Pluronics); cellulosic agents (e.g., carboxymethylcellulose); carbohydrates (e.g., sorbitol; mannitol); B vitamins (e.g., nicotinamide); xanthine derivatives; and alcohols (e.g., benzyl alcohol). Examples of acids to be used include the following: glucuronic;

galacturonic; fumaric; gentisic; acetic; citric and lactobionic. Types of antimicrobial agents that can be used are the following: phenylmercuric nitrate; thimerosal; benzethonium chloride; benzalkonium chloride; phenol; cresol; and chlorobutanol. An art-known local anesthetic (e.g., benzyl alcohol; NOVOCAINE.RTM.; lidocaine) may also be included.

DEPR:
An injectable composition comprising the osteoinductive extract and an oral composition comprising 1,25-dihydroxy Vitamin D.sub.3 for bone fracture repair is prepared by combining the following components utilizing conventional mixing techniques.

DEPR:
An injectable composition for bone fracture repair is prepared by combining the following components utilizing conventional mixing techniques.

DEPR:
A composition for inducing bone growth following reconstructive surgery is prepared by combining the following components utilizing conventional mixing techniques.

DEPR:
0.1 cc of the composition per cm.sup.2 of surface area of surgically reconstructed bone is deposited directly onto the bone surface.

DEPR:
A composition for accelerating the healing and providing a stronger bond between natural bone and an artificial prosthesis is prepared by combining the following components utilizing conventional mixing techniques.

DEPR:
0.1 cc of the composition per cm.sup.2 surface area of natural bone proximate to the prosthesis is deposited directly onto the natural bone.

DEPR:
After the patient is prepared using conventional periodontal surgical therapy 0.1 cc of the composition per exposed tooth is deposited into the surgery site. Soft tissue flaps are then sutured to close the surgical site. This treatment is useful for restoring alveolar and supporting bone in the periodontium lost by disease.

DEPR:
An injectable composition comprising the BMPs 2, 3, 4 and 5 and an oral composition comprising 1,25-dihydroxy Vitamin D.sub.3 for treatment of osteoporosis is prepared by combining the following components utilizing conventional mixing techniques.

DEPR:
A composition for inducing bone growth of a non-union fracture is prepared by combining the following components utilizing conventional mixing techniques. As used herein, "non-union

fracture" means a fracture that has failed to heal normally.

DEPR:
At the time of fracture reduction, a sufficient quantity of the above composition is deposited directly into the non-union site thereby filling in any bone deficit.

DETL:
Component of Composition Percent by Weight
BMP-2 0.04 25-hydroxy Vitamin D.sub.2 0.01 NaCl 0.09 Sterile water for injection q.s. 100.00

DETL:
Component Composition Percent by Weight
BMP-1 0.04 BMP-2 0.04 BMP-4 0.04 24,25-dihydroxy Vitamin D.sub.3 0.01 NaCl 0.90 Sterile water q.s. 100.00

DETL:
Component of Composition Percent by Weight
BMP-2 0.04 NaCl 0.90 Sterile water q.s. 100.00

DETL:
Component of Composition Percent by Weight
osteoinductive extract composition BMP-2 0.001 BMP-3 0.001 BMP-4 0.001 BMP-5 0.001 NaCl 0.900 Sterile water q.s. 100.000 1,25-dihydroxy Vitamin D.sub.3 composition 1,25-dihydroxy Vitamin D.sub.3 0.01 Corn starch 18.49 Lactose 63.00 Talc 18.00 Stearic acid 0.50 100.00

DETL:
Component of Composition Percent by Weight
BMPA 0.004 1,25-dihydroxy vitamin D.sub.3 0.010 Acid demineralized bone particles 90.000 NaCl 0.900 Sterile water for injection q.s. 100.000

CLPR:
1. A method of generating new bone growth in a mammal in need of such treatment comprising administrating to the mammal a bone morphogenetic protein (herein, "BMP") in combination with a Vitamin D compound, wherein the BMP is BMP-2 or BMP-4, and wherein:

CLPR:
2. The method of claim 1 wherein the BMP is BMP-2 and the Vitamin D compound is 1,25 dihydroxy Vitamin D.sub.3.

CLPR:

3. The method of claim 2 wherein about 500 ng of BMP-2 is administered in combination with about 6 ng of 1,25 dihydroxy Vitamin D.sub.3.

CLPR:
4. The method of claim 2 wherein about 1000 ng of BMP-2 is administered in combination with about 6 ng of 1,25 dihydroxy Vitamin D.sub.3.

CLPR:
9. A composition for generating new bone growth in a mammal in need of such treatment, the composition comprising:

CLPR:
10. The composition of claim 9 wherein the BMP is BMP-2 and the Vitamin D compound is 1,25 dihydroxy Vitamin D.sub.3.

CLPR:
11. The composition of claim 10 wherein about 500 ng of BMP-2 is administered in combination with about 6 ng of 1,25 dihydroxy Vitamin D.sub.3.

CLPR:
12. The composition of claim 10 wherein about 1000 ng of BMP-2 is administered in combination with about 6 ng of 1,25 dihydroxy Vitamin D.sub.3.

CLPV:
a. when the bone morphogenetic protein is BMP-2, from about 500 ng to about 1000 ng BMP-2 is administered in combination with about 6 ng of the Vitamin D compound; and

CLPV:
b. when the bone morphogenetic protein is BMP-4, about 62.5 ng BMP-4 is administered in combination with about 6 ng of the Vitamin D compound.

CLPV:
b. a bone morphogenetic protein (herein, "BMP"), wherein the BMP is BMP-2 or BMP-4; and

CLPV:
i. when the bone morphogenetic protein is BMP-2, from about 500 ng to about 1000 ng BMP-2 is administered in combination with about 6 ng of the Vitamin D compound; and

CLPV:
ii. when the bone morphogenetic protein is BMP-4, about 62.5 ng BMP-4 is administered in combination with about 6 ng of the Vitamin D compound.

ORPL:
"Bone Morphogenic Proteins and Vitamin D", Nutrition Reviews, vol. 47, pp. 364-366 (1989).

ORPL:
Sampath, T.K., S. Wientroub and A.H. Reddi, "Extracellular

Matrix Proteins Involved in Bone Induction Are Vitamin D Dependent", Biochemical and Biophysical Communications, vol. 124, No. 3, pp. 829-835 (Nov. 1984).

ORPL:
Turner, R.T., J. Farley, J.J. Vandersteenhoven, S. Epstein, N.H. Bell and D.J. Baylink "Demonstration of Reduced Mitogenic and Osteoinductive Activities in Demineralized Allogeneic Bone Matrix from Vitamin D-deficient Rats", The Journal of Clinical Investigation, Inc., vol. 82, pp. 212-217 (Jul. 1988).

ORPL:
Underwood, J.L. and H.F. DeLuca, "Vitamin D is Not Directly Necessary for Bone Growth and Mineralization", American Journal of Physiology, vol. 246, pp. E493-E498 (1984).

ORPL:
Wang, E.A., V. Rosen, J.S. D'Alessandro, M. Bauduy, P. Cordes, T. Harada, D.I. Israel, R.M. Hewick, K.M. Kerns, P. LaPan, D.P. Luxenberg, D. McQuiid, I.K. Moutsatsos, J. Nove and J.M. Wozney, "Recombinant Human Bone Morphogenetic Protein Induces Bone Formation", Wang, Proc. Natl. Acad. Sci. USA, vol. 87, pp. 2220-2224 (Mar. 1990).

ORPL:
Weintroub, S. and A.H. Reddi, "Vitamin D Metabolites and Endochondral Bone Development", Int. Congr. Ser.-Excerpta Med., vol. 589, pp. 211-217 (1991).

ORPL:
Wozney, J.M., V. Rosen, A.J. Celeste, L.M. Mitsock, M.J. Whitters, R.W. Kriz, R.M. Hewick and E.A. Wang, "Novel Regulators of Bone Formation: Molecular Clones and Activities," Research Articles, Science, vol. 242, pp. 1528-1534 (Dec. 1988).

ORPL:
"Demonstration of Reduced Mitogenic and Osteoinductive Activities in Demineralized Allogeneic Bone Matrix from Vitamin D-deficient Rats", R.T. Turner et al., The Journal of Clinical Investigation, Inc., vol. 82, pp. 212-217 1988.

ORPL:
"Extracellular Matrix Proteins Involved in Bone Induction Are Vitamin D Dependent", T.K. Sampath et al., Biochemical and Biophysical Research Communications, vol. 124, pp. 829-835 1984.

ORPL:
"Vitamin D Is Not Directly Necessary for Bone Growth and Mineralization", J. L. Underwood & H. F. DeLuca, American Journal of Physiology, vol. 246, pp. E493-E498, (1984).

ORPL:
"Vitamin D Metabolites and Endochondral Bone Development", Weintroub, S. and A. H. Reddi, (1991) Int. Congr. Ser. -Excerpta Med., vol. 589, pp. 211-217.

ORPL:
"Recombinant Human Bone Morphogenetic Protein Induces Bone Formation", Wang, et al., Proc. Natl. Acad. Sci. USA, vol. 87, pp. 2220-2224, Mar. 1990.

ORPL:
"Novel Regulators of Bone Formation: Molecular Clones and Activities," Wozney et al., Research Articles, Science, vol. 242, pp. 1528-1534, 1988.

WEST

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L8: Entry 10 of 10 File: USPT Jan 31, 1995

DOCUMENT-IDENTIFIER: US 5385887 A
TITLE: Formulations for delivery of osteogenic proteins

ABPL:
A composition is disclosed comprising a pharmaceutically acceptable admixture of an osteogenic protein; a porous particulate polymer matrix; an osteogenic protein-sequestering amount of blood clot; and a calcium sulfate hemihydrate-containing substance. Also disclosed are formulations of bone morphogenetic proteins with improved solubility and/or stability characteristics.

BSPR:
The subject invention relates to the field of osteogenic proteins and pharmaceutical formulations thereof. More particularly, the subject invention involves pharmaceutical formulations designed to sequester osteogenic protein in situ for a time sufficient to allow the protein to induce cartilage and/or bone formation.

BSPR:
Osteogenic proteins are those proteins capable of inducing, or assisting in the induction of, cartilage and/or bone formation. Many such osteogenic proteins have in recent years been isolated and characterized, and some have been produced by recombinant methods. For example, so-called bone morphogenic proteins (BMP) have been isolated from demineralized bone tissue (see e.g. Urist U.S. Pat. No. 4,455,256); a number of such BMP proteins have been produced by recombinant techniques (see e.g. Wang et al. U.S. Pat. No. 4,877,864 and Wang et al. U.S. Pat. No. 5,013,549); a family of transforming growth factors (TGF-.alpha. and TGF-.beta.) has been identified as potentially useful in the treatment of bone disease (see e.g. Derynck et al., EP 154,434); a protein designated Vgr-1 has been found to be expressed at high levels in osteogenic cells (see Lyons et al. (1989) Proc. Nat'l. Acad. Sci. USA 86, 4554-4558); and proteins designated OP-1, COP-5 and COP-7 have purportedly shown bone inductive activity (see oppermann, et al. U.S. Pat. No. 5,001,691).

BSPR:
Various attempts have been made at developing formulations designed to deliver osteogenic proteins to a site where induction of bone formation is desired. For example, certain polymeric matrices such as acrylic ester polymer (Urist, U.S.

Pat. No. 4,526,909) and lactic acid polymer (Urist, U.S. Pat. No. 4,563,489) have been utilized, but these formulations do not sequester the osteogenic protein for a time sufficient to optimally induce bone formation, and further have been found to erode too slowly for optimal bone formation.

BSPR:
A biodegradable matrix of porous particles for delivery of an osteogenic protein designated as OP is disclosed in Kuberasampath, U.S. Pat. No. 5,108,753. While U.S. Pat. No. 5,108,753 discloses that a successful carrier for OP must bind the protein, act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the protein from nonspecific proteolysis, no formulations are suggested which contain components that specifically sequester the OP at the site where bone formation is desired.

BSPR:
Ron et al., U.S. Pat. No. 5,171,579 discloses that the average surface area per porous particle is critical to optimize bone formation.

BSPR:
Okada et al., U.S. Pat. No. 4,652,441, U.S. Pat. No. 4,711,782, U.S. Pat. No. 4,917,893 and U.S. Pat. No. 5,061,492 and Yamamoto et al., U.S. Pat. No. 4,954,298 disclose a prolonged-release microcapsule comprising a polypeptide drug and a drug-retaining substance encapsulated in an inner aqueous layer surrounded by a polymer wall substance in an outer oil layer. Although bone morphogenic protein is listed as a polypeptide capable of such a formation, microencapsulation of osteogenic proteins prevents controlled release of such protein sufficient for optimal bone formation.

BSPR:
Yamazaki et al., Clin. Orthop. and Related Research, 234:240-249 (1988) disclose the use of implants comprising 1 mg of bone morphogenetic protein purified from bone and 5 mg of Plaster of Paris. U.S. Pat. No. 4,645,503 discloses composites of hydroxyapatite and Plaster of Paris as bone implant materials.

BSPR:
Collagen matrices have also been used as delivery vehicles for osteogenic proteins (see e.g. Jeffries, U.S. Pat. No. 4,394,370), but collagen frequently causes undesirable antigenic reactions in patients. Therefore, there remains a need for a pharmaceutical formulation capable of sequestering osteogenic proteins at a site where induction of bone formation is desired for a time sufficient to allow safe, effective induction of such bone formation.

BSPR:
Applicant has further found, surprisingly, that an improved formulation of BMPs, particularly BMP-2, which has improved solubility and stability characteristics, can be achieved using

a composition of glycine, sucrose, and glutamic acid hydrochloride, at a pH of less than 6.0. In a preferred embodiment of the invention, this formulation comprises about 2.5% glycine (g/100 ml (w/v)), about 0.5% sucrose (w/v), about 5 mM glutamic acid hydrochloride (about 0.1% w/v), and about 0.01% (w/v) polysorbate 80, at a pH of about 4.5.

BSPR:
The compositions of the present invention are useful for the preparation of formulations of osteoinductive proteins which can be used, among other uses, to promote the formation of cartilage and/or bone, for repair of tissue damage and fractures.

BSPR:
In U.S. Pat. No. 5,171,579, it is disclosed that osteogenic proteins can be sequestered at a site where bone inducing activity is desired using autogenous blood, without using antifibrinolytic agents, provided that a porous particulate polymer matrix is incorporated into the formulation. To reduce the preparation time and improve the above formulation's handling characteristics, Applicants have surprisingly found that it is desirable to add a calcium sulfate hemihydrate-containing substance (CSHS). The CSHS is preferably either pure calcium sulfate hemihydrate (CaSO.sub.4 *1/2H.sub.2 O), also known as Plaster of Paris (POP), or a mixture of POP and hydroxyapatite (POP:HA). Adding a CSHS reduces setup time and provides improved moldability and consistency of the resulting formulation.

BSPR:
The osteogenic proteins useful in the practice of the subject invention are well known to those skilled in the art and include those discussed above. The preferred osteogenic proteins for use herein are those of the BMP class identified as BMP-1 through BMP-10 in U.S. Pat. No. 4,877,864; U.S. Pat. No. 5,013,649; WO 90/11366 published Oct. 4, 1990; WO 91/18098 published Nov. 28, 1991; WO 93/00432, published Jan. 7, 1993 and U.S. Ser. No. 08/061,695, filed May 12, 1993. The disclosure of the above publications are hereby incorporated by reference. The most preferred is BMP-2, the full length cDNA sequence of which is described in detail in the '649 patent. Of course, combinations of two or more of such osteogenic proteins may be used, as may fragments of such proteins that also exhibit osteogenic activity. Such osteogenic proteins are known to be homodimeric species, but also exhibit activity as mixed heterodimers. Heterodimeric forms of osteogenic proteins may also be used in the practice of the subject invention. BMP heterodimers are described in WO93/09229, the disclosure of which is hereby incorporated by reference. Recombinant proteins are preferred over naturally occurring isolated proteins. The amount of osteogenic protein useful herein is that amount effective to stimulate increased osteogenic activity of infiltrating progenitor cells, and will depend upon the size and nature of defect being treated as discussed in more detail below, such amounts being orders of magnitude less than the amount of porous particulate polymer matrix employed, generally

in the range of 1-50 .mu.g of protein for each 10 mg of porous particulate polymer matrix employed and more preferably in the range of 0.5-10 .mu.g protein for each milligram of polymer matrix employed (assuming 0.2 g/cc density).

BSPR:
The osteogenic proteins can be utilized in the form of a pharmaceutically acceptable solution (including reconstitution from a lyophilized form). It is optimal to solubilize the osteogenic protein at concentrations of at least about 1 mg/ml, preferably about 2 to 8 mg/ml, so that a pharmaceutically effective amount of protein can be delivered without undue volumes of carrier being necessary. For some applications, concentrations above 2 mg/ml may be desirable. Amino acids having a net positive charge (e.g. net 1+ species such as arginine, histidine, lysine and the ethyl esters of glycine and beta-alanine), preferably a net 2+ charge (e.g. the ethyl ester of histidine, the methyl esters of lysine and arginine, and agmatine), are useful in this regard. Amino acids having a net zero charge are useful in this regard provided that the positive charge of the compound is sufficiently distant (at least 2-3 CH.sub.2 units away) from the neutralizing negative charge (e.g. net neutral species such as gamma-amino butyric acid, beta-amino propionic acid, and glycine-glycine dipeptide). Other solubilizing agents useful herein include poly(sorbate), dextran sulfate, guanidine, heparin, sodium chloride, glutamic acid hydrochloride, acetic acid and succinic acid. For use in solubilizing dimeric BMP, such as BMP-2, 3, 4, 5, 6, 7, 8, 9 and 10 and heterodimers of BMPs such as BMP-2/6 and BMP-2/7, preferred solubilizing agents include arginine and histidine (including esters thereof) and glutamic acid hydrochloride.

BSPR:
Glutamic acid hydrochloride (HCl) is the preferred solubilizing agent. It is used in concentrations of about 1 to about 20 mM (about 0.02 to about 0.37% w/v), preferably about 5 to about 10 mM (about 0.1 to about 1.8% w/v), and at a pH of less than about 6.0, preferably about 3.5 to about 5.25, preferably about 4.5. The composition also includes about 0.1 to about 5.0% (w/v), preferably about 0.5 to about 2.5%, of a sugar; most preferably about 0.5% sucrose; and about 0.5 to about 10.0% (w/v) glycine, preferably about 2.0 to about 2.5%. In order to prevent the formation of particulates, the glutamic acid hydrochloride composition may optionally include about 0.01 to about 0.1% (w/v) of a non-ionic surfactant, such as a polyoxyester, for example polysorbate 80, polysorbate 20 or Pluronic F-68. The sucrose/glycine ratio can be varied to provide moisture content and bulk characteristics to the composition. Other components may be substituted in the compositions of the present invention. For example, in place of glutamic acid, a mono- acid with pKa close to 4.5 can be used, such as acetic acid. Di- acid like succinic acid can be used, but at a lower concentration (about 1 mM preferred, rather than about 5 mM). When glutamic acid hydrochloride is used, either glutamic acid/hydrochloride or mixtures of glutamic acid and HCl may be used. Glutamic acid hydrochloride may also be used

in hydrated form, such as glutamic acid hydrochloride monohydrate. With minor modifications, glutamic acid or glutamates, for example sodium glutamate, may be used in place of glutamic acid hydrochloride. Glycine may be replaced in whole or in part by other amino acids with similar charge. In addition, other optional components may be added such as albumin, glycerol, mannitol and other sugars. Thus, the present invention includes compositions employing substituted components, as described above, for example, compositions using glutamic acid or sodium glutamate in place of glutamic acid hydrochloride. Other modifications will be apparent to those skilled in the art and are also encompassed by the present invention.

BSPR:
The above formulations can be reproducibly lyophilized in less than 40 hours, with moisture level well controlled. The formulations provide good storage stability at a variety of temperatures. The above formulations further provide higher solubility of BMP, providing support for administration of higher doses of BMP, and show good compatibility with the devices described above. With minor variations and modifications within the present invention, the above formulation may be used to prepare solutions of higher concentrations of at least about 4 mg/ml of BMP-2 or other BMPs.

BSPR:
The porous particulate polymer matrix component useful in the practice of the subject invention is a polymeric material that can be formed into porous particles as described below thereby providing in situ scaffolding for the osteogenic protein, while having biodegradable properties allowing for replacement by new bone growth. Examples are polymers of amino acids, orthoesters, anhydrides, propylene-co-fumarates, or a polymer of one or more .alpha.-hydroxy carboxylic acid monomers, (e.g. .alpha.-hydroxy acetic acid (glycolic acid) and/or .alpha.-hydroxy propionic acid (lactic acid)). The latter can be employed in its d- or 1-form, or as a racemic mixture, the racemic mixture being preferred. When a copolymer of lactic acid and glycolic acid is employed (PLGA), the molar ratio of monomers can range from 1:99 to 99:1 depending upon the desired bioerosion lifetime which in turn depends upon the clinical indication being addressed, as more than 50% of either monomer gives longer bioerosion lifetime (slower biodegradation). The molecular weight of the polymer can range from about 1,000 to 100,000 (relative to polystyrene in CHCl.sub.3) with 30,000-50,000 being preferred when a 50:50 copolymer is employed. In general, the higher the molecular weight, the slower the biodegradation.

BSPR:
U.S. Pat. No. 5,171,579 discloses that the average surface area per porous particle is critical to optimize bone formation. Specifically, porous particles useful in bone formation according to the present invention should have an average surface area of from about 0.02 to 4 m.sup.2 /g. WO 93/06872

further discloses that it is possible to produce porous particles having the desired surface area by introducing a "porosigen" (composition capable of imparting porosity by increasing particle surface area) into the solution used to produce the porous particles. The disclosure of the above publications are hereby incorporated by reference. It is also possible to control the bioerosion rate by subjecting the porous particles to sterilizing doses of .gamma. radiation. The higher the .gamma. radiation dose, the faster the bioerosion. Particles useful herewith have a porosity such that the surface area of the particles is increased about 2-250 fold over the surface area of non-porous particles of comparable size.

BSPR:
In yet another embodiment of the present invention, the formulation comprises suitable mixtures of osteogenic protein and calcium sulfate hemihydrate-containing substance. Preferred compositions of such formulations comprise approximately 0.5 to 2 grams, preferably 1 gm, of osteogenic protein per approximately 10 to 20 grams, preferably 12 grams of CSHS in approximately 3 ml of H.sub.2 O. For example, the osteogenic protein can be delivered using 0.5 ml of a 2 mg/ml BMP-2 solution, or 0.25 ml of a 4 mg/ml BMP-2 solution. In this embodiment, the CSHS provides a structural matrix function, an osteoconductive matrix, and a protein sequestering function.

BSPR:
The formulations of the subject invention provide malleable implants that allow therapeutically effective amounts of osteoinductive protein to be delivered to an injury site where cartilage and/or bone formation is desired. Such an implant may be used as a substitute for autologous bone graft in fresh and non-union fractures, spinal fusions, and bone defect repair in the orthopaedic field; in cranio/maxillofacial reconstructions; for prosthesis integration, especially as a surface coating to improve fixation of prosthetic implants such as hydroxylapatite coated prostheses; in osteomyelitis for bone regeneration; and in the dental field for augmentation of the alveolar ridge and periodontal defects and tooth extraction sockets. When used to treat osteomyelitis or for bone repair with minimal infection, the osteogenic protein may be used in combination with porous microparticles and antibiotics, with the addition of protein sequestering agents such as alginate, cellulosics, especially carboxymethylcellulose, diluted using aqueous glycerol. The antibiotic is selected for its ability to decrease infection while having minimal adverse effects on bone formation. Preferred antibiotics for use in the devices of the present invention include vancomycin and gentamycin. The antibiotic may be in any pharmaceutically acceptable form, such as vancomycin HCl or gentamycin sulfate. The antibiotic is preferably present in a concentration of from about 0.1 mg/mL to about 10.0 mg/mL.

BSPR:
The lower viscosity formulations may also be used as a percutaneous injection to accelerate healing of closed fractures. In certain of these uses, the compositions of the

subject invention may be used in combination with various bone cements, including erodible bone cements such as poly(propylene-co-fumarate) and certain hydroxyapatite cements. Also, certain of these uses will utilize bioerodible hardware such as erodible plates, screws, etc. As alluded to above, the dosage regimen will be determined by the clinical indication being addressed, as well as by various patient variables (e.g. weight, age, sex) and clinical presentation (e.g. extent of injury, site of injury, etc.). In general, the dosage of osteogenic protein will be in the range of from about 10 to 1000 .mu.g, preferably from about 10 to 100 .mu.g.

BSTL:
TABLE 1 _____ a. 4.0 mg/ml formulation BMP-2 4.0 mg/ml (11.42% wt) glutamic acid HCl 0.918 mg/ml (2.62% wt) glycine 25 mg/ml (71.39% wt) sucrose 5 mg/ml (14.28% wt) polysorbate 80 0.1 mg/ml (0.29% wt) b. 2.0 mg/ml formulation BMP-2 2.0 mg/ml (6.06% wt) glutamic acid HCl 0.918 mg/ml (2.78% wt) glycine 25 mg/ml (75.72% wt) sucrose 5 mg/ml (15.14% wt) polysorbate 80 0.1 mg/ml (0.30% wt)

DEPR:
Recombinantly produced BMP-2 is solubilized (2 mg/ml) in a composition of 2.5% (w/v) glycine, 0.5% (w/v) sucrose, 0.01% (w/v) polysorbate 80 and 5 mM (about 0.092% w/v) glutamic acid HCl at pH=4.5.

DEPR:
The compositions are reconstituted at 2 weeks, 1 month, 3 months and 4.5 months after lyophilization and studied for reconstitution time, particulate formation, pH after reconstitution, % moisture content, concentration of BMP-2 after reconstitution, % aggregate formation and specific activity in the W-20 bioassay for BMP activity.

DEPR:
Lyophilized recombinant human BMP-2 (rhBMP-2) (2 mg in buffer) is reconstituted with 1 ml sterile water for injection (WFI). 0.5 ml of the rhBMP-2 solution (1 mg) is drawn into a 3 ml syringe using a 22 g needle, which is then removed.

DEPR:
Use of the W-20 bone marrow stromal cells as an indicator cell line is based upon the conversion of these cells to osteoblast-like cells after treatment with a BMP protein [Thies et al, Journal of Bone and Mineral Research, 5:305 (1990); and Thies et al, Endocrinology, 130:1318 (1992)]. Specifically, W-20 cells are a clonal bone marrow stromal cell line derived from adult mice by researchers in the laboratory of Dr. D. Nathan, Children's Hospital, Boston, Mass. Treatment of W-20 cells with certain BMP proteins results in (1) increased alkaline phosphatase production, (2) induction of PTH stimulated cAMP, and (3) induction of osteocalcin synthesis by the cells. While (1) and (2) represent characteristics associated with the osteoblast phenotype, the ability to synthesize osteocalcin is a phenotypic property only displayed

by mature osteoblasts. Furthermore, to date we have observed conversion of W-20 stromal cells to osteoblast-like cells only upon treatment with BMPs. In this manner, the in vitro activities displayed by BMP treated W-20 cells correlate with the in vivo bone forming activity known for BMPs.

DEPR:
10 uL of 8 mg/mL rhBMP-2 formulation (in buffer as described for the 2.0 mg/ml formulation at pages 6-7 above) or 10 uL of control (buffer without BMP-2) is added to a sterile eppendorf tube containing 37.2 mg (0.200 mL) 50:50 PLGA porous particles.

DEPR:
After 14 days, the rats are sacrificed and each animal is evaluated for bone formation.

DEPR:
A critical-size defect (5 mm) is surgically created in the mid-diaphysis of the left femur of each of 56 male Long Evans retired breeder rats (450-550 grams), by affixing a pre-drilled polyethylene plate to the anterior portion of the femur and excising a segment of bone with a carbide dental drill. A bioerodible implant is prepared by mixing rhBMP-2 (in varying amounts), PLGA porous paticles, calcium sulfate hemihydrate-containing substance and venous rat blood and allowing the blood to clot to form a moldable implant. Eight groups of seven animals each are implanted as follows: 0 .mu.g rhBMP-2; 0.93 .mu.g rhBMP-2; 3.1 .mu.g rhBMP-2; and 9.3 .mu.g rhBMP-2.

DETL:
TABLE 4 Alkaline Phosphatase Values for W-20 Cells Treating with BMP-2 BMP-2 concentration Absorbance Reading umoles substrate ng/ml 405 nmeters per hour 0 0.645 0.024 1.56 0.696 0.026 3.12 0.765 0.029 6.25 0.923 0.036 12.50 1.121 0.044 25.0 1.457 0.058 50.0 1.662 0.067 100.0 1.977 0.080

DETL:
TABLE 5 Average Bone Test Group Score
Negative control (no B MP) 0 Positive control BMP(10 ug/100 uL) 2.75 Vmycin 10.0 mg/100 uL BMP(40 ug/100 uL) 4 Vmycin 5.0 mg/100 uL BMP(40 ug/100 uL) 4.8 Vmycin 1.0 mg/100 uL BMP(40 ug/100 uL) 3.5 Vmycin 0.1 mg/100 uL BMP(40 ug/100 uL) 4.2 Gmycin 10.0 mg/100 uL BMP(40 ug/100 uL) 3.4 Gmycin 5.0 mg/100 uL BMP(40 ug/100 uL) 3.1 Gmycin 1.0 mg/100 uL BMP(40 ug/100 uL) 4.1 Gmycin 0.1 mg/100 uL BMP(40 ug/100 uL) 3.8
Bone Histology Scoring Code: 0 = No bone 1 = Bone in 10-20% of section 2 = Bone in 20-40% of section 3 = Bone in 40-60% of section 4 = Bone in 60-80% of section 5 = Bone in 80-100% of section Controls contained no antibiotic Vmycin = vancomycin HCl Gmycin = gentamycin sulfate BMP = recombinant human BMP2

CLPR:
6. A composition according to claim 5 wherein the lyophilized formulation comprises relative weight amounts of about 4.0 mg/ml of BMP-2; about 0.918 mg/ml of glutamic acid hydrochloride; about 25 mg/ml of glycine; about 5 mg/ml of sucrose; and optionally about 0.1 mg/ml of polysorbate 80.

CLPR:
7. A composition according to claim 5 wherein the lyophilized formulation comprises relative weight amounts of about 2.0 mg/ml of BMP-2; about 0.918 mg/ml of glutamic acid hydrochloride; about 25 mg/ml of glycine; about 5 mg/ml of sucrose; and optionally about 0.1 mg/ml of polysorbate 80.